Perspective

A sterile-female technique proposed for control of *Striga hermonthica* and other intractable weeds: advantages, shortcomings and risk management

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Abstract

Weeds have posed intractable challenges to farmers since the dawn of agriculture. This article describes in detail a proposed control strategy based on the introduction of genes conferring female sterility into the genome of an intractable target weed. Spread of these genes through target populations via pollen would be facilitated by their incorporation within active transposable elements. Advantages (e.g. self-dissemination, self-proliferation, target specificity) and shortcomings (e.g. high cost, long project incubation period, limited range of possible targets) of this strategy are discussed in depth, as are assessment and management of its attendant biological and ecological risks, such as the risk of introduced genes spreading to non-target species. The parasitic weed *Striga hermonthica* (Del.) Benth. is examined as a potential target.
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1 INTRODUCTION

Striga hermonthica (Del.) Benth. and many other parasitic plants are intractable weeds, in spite of attempts to control them by biological, chemical or cultural means.[1] Examples of proposed strategies for control of parasitic weeds include: cultural practices such as intercropping[2,3] or rotation with non-host plants;[4] treatment with chemical herbicides[5] or natural[6,7] and enhanced[8,9] bioherbicides; combinations of chemical and bioherbicides;[10] spurious induction of germination;[11] use of herbicide-resistant crops to facilitate such treatments;[12] and use of other crop-expressed strategies such as allelopathy,[13] natural[14,15] or transgenic[16] forms of host-plant resistance or RNA interference.[17] Most control strategies proposed for parasitic weeds, including those listed above, are designed to temporarily suppress a target weed population during the time that it would directly threaten a given crop in a given treatment area.

Weed control can also focus on long-term reduction of the overall population density of the target to subeconomic levels, as with the release of herbivorous[18] or pathogenic[19] classical biological control agents that establish, proliferate and disseminate throughout the area of weed infestation, are highly target specific and inflict enough damage to reduce target populations.[20,21] One advantage of such a self-proliferating, self-disseminating strategy is that continuous, long-term control of the weed can be achieved without incumbent labour, germplasm or treatment imperatives on the farmer.[21] The net result is reduced pressure from the target weed owing to the independent activity of the classical biocontrol agent. Once target weed populations have been reduced to economically or ecologically benign levels, agent populations also subside, in accordance with their obligate relationship to the target.[20] In cases where such long-term population reduction strategies do not provide stand-alone control, they may still complement inundative or cultural control strategies by reducing the frequency with which such treatments are necessary.

Classical biocontrol, when it is effective, is among the most elegant and economical weed control options. However, it is not applicable to all target weeds, particularly when the target is weedy in its native range (as with *S. hermonthica*). Also, because of its high initial cost and long incubation period, classical biocontrol has often been practised as a 'last line of defence' against intractable weeds. Data compiled from 76 classical weed biocontrol programmes in Australia, Hawai'i and South Africa, covering more than a century,[20,22] indicate that, while overall success was very high (23 : 1 return on investment, including failed programmes), control was not achieved (i.e. net loss or zero return on investment) for approximately one-third of targeted weeds. Thus, alternative solutions are needed for many targets, including some for which classical biocontrol was originally considered promising.

A novel strategy has been proposed for control of certain weed species, including parasitic weeds such as *S. hermonthica*, that seeks to emulate some of the characteristics of a successful classical biocontrol agent, including self-proliferation, self-dissemination, high target specificity, long-term target reduction and reduced agent presence after control is achieved.[23] The proposed strategy seeks to introduce female sterility in the target weed and spread

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this trait through noxious populations of the target. This article will summarise this sterile-female technique and discuss its advantages and shortcomings, as well as management of its attendant biological and ecological risks.

2 STERILE-FEMALE TECHNIQUE

The sterile-female technique is based on the introduction of genes into the target species genome that would cause female sterility while maintaining male fertility. It is designed to act without induction and spread through a weed population via pollen from female-sterile target plants to conspecific wild-type target plants, which would serve as the female parents. Female-sterile genes would be introduced into the target genome packaged within an active transposable element to facilitate its spread through the target population. A similar concept, known as 'daughterless', was proposed for control of introduced carp in Australia,[24] although the biology of that vertebrate system is obviously quite different from a plant system and it does not propose the use of a transposon-enhanced vector.

A female-sterility gene construct should contain certain essential components, in tandem orientation. These include: a gene such as *barnase*[25] that destroys the tissue in which it is expressed, driven by a promoter that is active only in a female reproductive organ;[26] a visual marker gene (e.g. leaf or flower pigmentation) for rapid identification in the laboratory and field; an inducible[27] 'kill switch' that would render the female-sterile plant susceptible to an otherwise benign surface treatment so that it could be selectively killed if necessary (analogous to the *kev* genes proposed by Gressel and Levy[28] for control of *S. hermonthica*); and a second inducible gene that would deactivate the female-sterility trait in order to allow prerelease interbreeding and increase of female-sterile seed. An important aspect of the female-sterile gene construct is its incorporation, if possible, within an active transposable element prior to transformation.[29] This would allow continual duplication and dissemination of the construct within the genomes of female-sterile target plants, maximising the likelihood that copies will be present in all gametes and therefore pass to all progeny of any

female-sterile × *wild-type* cross. This transposon-vectoring system, dubbed TAC-TICS (Transposon Armed Cassettes – Targeted Insect Control Strategy), has been demonstrated to bring about rapid spread of a functional allele through a naive insect population.[30] Plant transposons such as Ac/Ds[31] have been proposed to achieve this end in plant systems.[28] Other possible components of the tandem construct, e.g. to prevent impact on non-target species, are discussed below.

The female-sterility construct would replicate during meiosis and be sexually transmitted in pollen; therefore, spread of the construct would be highly specific to the target species. However, attention would be necessary to the possibility of hybridisation between the target and other closely related species (see Section 4 on risk assessment and management). Since female-sterile plants would produce only pollen and require conspecific wild-type plants as 'surrogate mothers', they would be sexually inert, incapable of reproduction by themselves.

The underlying goal of the sterile-female technique is replacement of the target species seed bank with conspecific female-sterile seed. Female-sterile target plants would compete with wild-type target plants to pollinate a fixed number of wild-type target pistils, producing female-sterile seed instead of wild-type seed when successful. Germination of seed from such a cross would produce more female-sterile plants that would once again compete with wild-type plants for pollination opportunities. Repeated iterations of this cycle would yield steadily increasing proportions of female-sterile target weed seed in the seed bank, compared with conspecific wild-type target weed seed, and thus increasing proportions of female-sterile plants in subsequent generations. Ultimately, the last of the wild-type target seed would germinate and be pollinated by a surrounding sea of female-sterile plants, producing only female-sterile seed. In subsequent generations, only female-sterile target weed seed would remain in the seed bank, giving rise exclusively to female-sterile target plants. No further target seed could be produced, and eventually the remaining female-sterile target seed in the soil would germinate and perish. Ideally, they would be replaced by non-weedy, native flora (Fig. 1). Thus, the use of the sterile-female technique for weed control
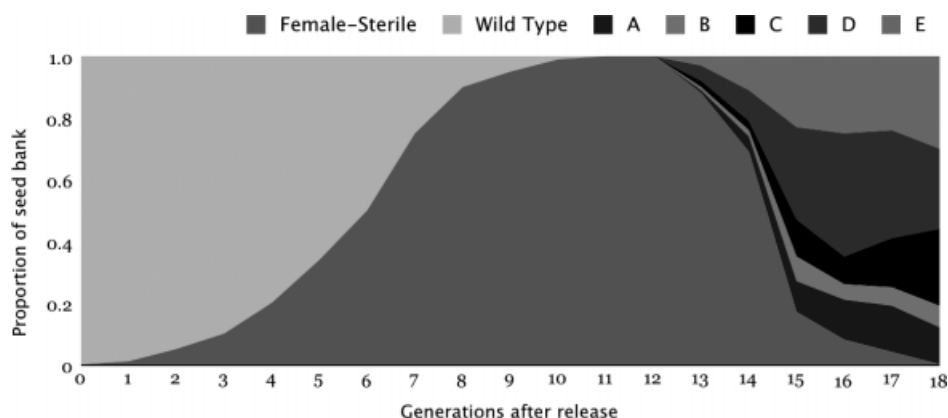

Figure 1. Colonisation of seed bank by female-sterile seed. With successive post-release generations, female-sterile seed is expected to make up an increasing proportion of the target weed species seed bank as pollen from female-sterile plants competes with wild-type target pollen to fertilise decreasing numbers of wild-type pistils. Following complete replacement of wild-type target seed by female-sterile seed, the target population would crash as female-sterile seed germinate but do not reproduce. Other plant species (designated A to E here) would then have the opportunity to colonise the niche formerly dominated by the invasive target weed. Data shown are hypothetical. Parameters used to model such colonisation could include: the dynamics of the transposon in the target weed genome; the density, sex ratio (if applicable) and relative frequency of allogamy, autogamy and apomixis in the target population; the reproductive biology of the target; the average distance travelled by target pollen and seed in one generation; and the longevity of the target seed in the soil relative to generation time. Certain parameters (e.g. the presence of autogamy or apomixis in the target population) could preclude complete displacement of wild-type seed.

would represent only a temporary presence of transgenic plants in the field.

3 ADVANTAGES AND SHORTCOMINGS

As a self-sustaining biologically based strategy, sterile-female technique incorporates many of the advantages of classical biocontrol, including self-proliferation, self-dissemination, high host specificity, energy efficiency, biodegradability and reduced presence after control is achieved.[21] Biotic[32–34] or abiotic[35–37] environmental factors can limit or prevent establishment of classical biocontrol agents, but since female sterility would be incorporated within the target species itself, it should be active in any environment in which the wild-type plant can grow.

Spread of the female-sterile construct through the target population could be modelled on the basis of the dynamics of the transposon in the target weed genome (namely the average number of copies made per generation and the upper limit of copy number within the host genome), the density of the target population, the reproductive biology of the target (e.g. annual versus perennial, monoecious versus dioecious, occurrence of asexual reproduction), sex ratio (if applicable), the relative frequency of allogamy (i.e. outcrossing) versus autogamy (self-pollination) or apomixis in the target population, the average distance travelled by the target pollen and seed in one generation and the longevity of the target seed in the soil relative to generation time.

In the case of targets for which an effective transposon-vectoring system would not be possible, it would be desirable to maximise the penetration of the female-sterile allele through a target weed population by performing several generations of prerelease interbreeding between female-sterile plants derived from independent transformation events. This would produce lines with multiple, unlinked copies of the female-sterile allele. The persistence of the female-sterile allele in the target population could then be modelled, based on the number of loci in the release generation and various characters of the target population (as discussed above). However, without a transposon-vectoring system, this persistence would ultimately be finite owing to continuous crossing to wild-type plants. Thus, in such a case, repeated releases of female-sterile plants would likely be necessary to achieve control.

Important shortcomings of this proposed strategy include the large cost in time, labour and resources that is likely to be necessary, and the reliance on allogamy for spread of female sterility. Avoidance of non-target effects is a very important issue (see Section 4), and, while risks can be managed and mitigated, biological systems tend to be unpredictable, and therefore unforeseen effects cannot be ruled out. The relative likelihood and impact of these shortcomings should be estimated and weighed against the expected benefit from control of the weed.

Parasitic plant seeds may lie dormant in the soil until stimulated to germinate by the presence of host roots.[38] Female-sterile target seed should react to such stimuli identically to wild-type target seed, ensuring that female-sterile plants would always be present to compete for pollination opportunities when wild-type plants are present. This should be true even when seed numbers become limiting, when several years pass between suitable germination conditions or in non-cropping situations where the target weed is parasitising wild plant species.

A possible shortcoming of the sterile-female technique would arise if the female-sterile construct presented a metabolic cost to the plant that reduced its competitive ability with wild-type plants. However, only one gene in the construct, the visual marker gene, would be constitutively expressed, and this expression could be directed to certain tissues, if necessary. The other genes in the construct would only be transiently expressed (in the case of the female-sterility gene) or under induction by external stimuli not normally occurring in the field (in the case of the kill switch and the female-sterility deactivator). Another possible concern could be complications with gene silencing[39] owing to the presence of multiple copies of the female-sterility construct. This could be tested prior to release.

Owing to the reliance on allogamy in the target species in the sterile-female technique, selection for autogamy in the target population would be likely, causing a 'genetic bottleneck' in the resulting target population structure. The effect of this autogamous shift on the invasiveness of a normally highly allogamous target population would be difficult to predict and would likely be case specific. However, it has been proposed that allogamy is maintained in large, stable plant populations owing to pressure from pests,[40] since in the event of an epidemic or epizootic a wide variety of genotypes in a population would increase the probability of survival of at least one. Thus, shifting an invasive weed population from allogamy to autogamy may not completely eradicate it, but could render it highly susceptible to subsequent insect or pathogen outbreaks, whether naturally occurring or as biocontrol agents. Selection for selfing would also be particularly disadvantageous in many autopolyploid targets owing to inbreeding depression.[41]

4 RISK ASSESSMENT AND MANAGEMENT

The release of transgenic organisms into the environment presents clear risks, in particular the risk of gene flow to closely related species that are likely to be sympatric with released organisms. In the case of the sterile-female technique, female-sterile plants are expected to establish within the target population until control is achieved. Therefore, closely related, sympatric species should be tested for their susceptibility to hybrid fertilisation by pollen of the target species.[42] It seems unlikely that the female-sterile construct would confer a competitive advantage to a wild non-target plant, which is an important concern surrounding the release of transgenic crops containing genes that protect against pests or herbicides.[43] However, the opposite concern would be present with the sterile-female technique, since the female-sterile construct is designed to spread through populations and considerably reduce their densities. Components of the proposed construct, including the visual marker and the 'kill switch' (see Section 2), would aid in recognising and mitigating such spread respectively. However, additional traits designed to avert such interspecific gene flow would also be desirable. One possibility that is proposed here is to include an additional *barnase* gene in the construct that would be under the control of transcription factors that would only be active in the non-target genetic background.

Since short, *cis*-acting, regulatory sequences, commonly located in promoters, tend to be highly conserved in plants, promoters that are non-functional in the target species and functional in a closely related non-target species are likely to be rare, although functional variations are being sought.[44] However, many transcription factors affect gene regulation, in spite of being distant from the genes in question,[45] whether in *cis*- or *trans*-chromosomal orientation. In

addition, regulatory 'hot spots' have been detected that contain transcription factors influencing gene expression throughout the genome.[45] Protocols have been devised to detect variation in such regulatory activity,[46,47] and extensive intraspecific[48,49] and interspecific[50–52] variation in gene expression has been found in model organisms. Such variation is not necessarily associated with natural selection for positive functional adaptation,[53] although data indicate that, in closely related species, sex-linked gene expression diverges faster than expression in the rest of the transcriptome,[50] as might be expected from the sexual essence of the definition of species. In one model system,[52] 53 genes were found to be exclusively expressed in one species but appeared to be 'turned off' in another closely related species, including sex-linked genes.

Given such divergent expression between closely related species, it may be possible to express a *barnase* gene exclusively in the non-target background – which would be present in a *target* × *non-target* cross. For example, a *barnase* allele included in the female-sterility construct that is controlled by transcription factors from a male reproductive gene expressed exclusively in the non-target species would render *target* × *non-target* hybrid plants male sterile. Since they would also already be female sterile, this would prevent unwanted spread of the construct (Fig. 2). Indeed, any gene expressed exclusively in the non-target background prior to fertilisation (e.g. in germination, seedling or vegetative stage) could be used to this end, as long as a *barnase* in its place would effectively kill or sterilise a *target* × *non-target* hybrid plant. The genetic information required to build such a construct would be expensive to generate, as it would require the availability of ample genetic resources for both the target and non-target species. For some crop species, such genetic resources are already available, and this hybrid-prevention system could be useful in preventing escape of transgenes from crop species to their wild relatives.[42]

Other risks include the female-sterile construct becoming deactivated after release, perhaps by mutation or methylation. The risk of deactivation by mutation seems unlikely considering the number of loci that would be present owing to the effect of the transposon vector, although mutated copies might give rise to repressors that bind to promoters within the construct. Methylation of all copies of the construct might be possible, but should be detectable before release, e.g. by suppression of visual marker activity. In either case, a plant carrying a deactivated female-sterile construct would be expected to have a wild-type phenotype, since the construct would have been introduced into a wild-type background. Therefore, such a plant should pose no more risk than any of the wild-type plants in the invasive population into which it is released. Other risks include that to herbivores that feed on target plant tissue. However, since the goal of the sterile-female technique is to eradicate a target weed population, questions surrounding herbivory would ultimately become irrelevant.

The risk of escape of the female-sterile construct to a target species centre of origin, or the use of the sterile-female technique within a target species centre of origin, is predicated on the danger that the sterile-female technique could ultimately drive a target to extinction. A combination of precautionary measures and biological realities would preclude this danger. The visual marker and kill switch included in the construct are intended to provide means of both warning and mitigation of unwanted spread. As discussed above, there would be heavy selection for autogamy with the sterile-female technique, which would result in remnant self-pollinating populations of the target weed.
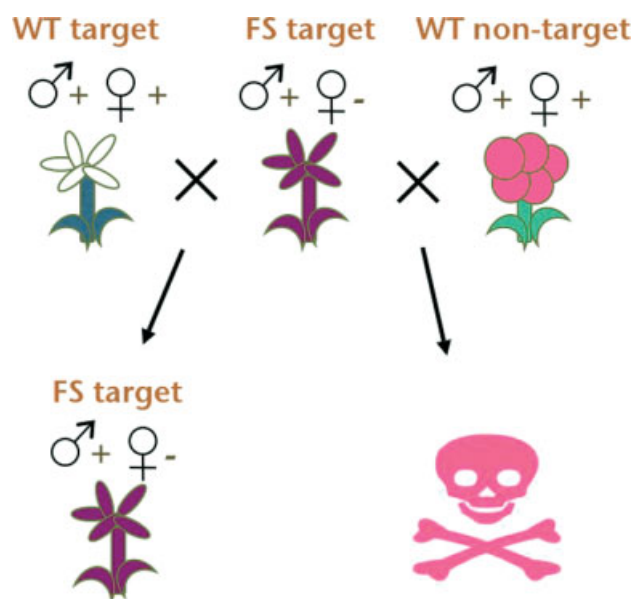

Figure 2. Prevention of interspecific hybridisation. Prevention of interspecific hybridisation may be attempted by incorporating a destructive gene (e.g. *barnase*) into the female-sterility construct under the control of transcription factors that are active in the genetic background of non-target species but not in that of the target species. Expression of this destructive gene should occur either in a preflowering development stage, to kill a hybrid plant, or in a male reproductive organ, in order to render the hybrid male sterile and prevent further interspecific spread of the female-sterility construct. WT = wild type; FS = female sterile.

Ultimately, seed or other germplasm of the target weed could be collected from its native range prior to release of female-sterile plants for preservation. Indeed, in the case of *S. hermonthica*, it is possible to imagine a world where this devastating weed could only be found in botanical gardens.

Risks posed by the sterile-female technique should be weighed against the expected benefit of controlling the target weed by comparison with an alternative practice or perpetuation of the status quo.[54] Such risk benefit assessments are a primary tool of regulatory authorities and apply to all control strategies, whether chemically or biologically based.

5 POTENTIAL TARGETS

As a strategy that relies on spread between plants via pollen, the sterile-female technique would only be feasible against allogamous weed species that reproduce or spread primarily by seed. The target species must also be amenable to transformation and preferably be susceptible to transposon activity. In addition, because of the investments in time and resources that would be necessary to carry out such a project, targets selected for such a project would tend to be truly intractable weeds for which other control measures had already failed, were not suitable or were not cost effective. Ecological considerations such as lack of hybridisation with sympatric, close relatives and lack of proximity of the release site to the centre of origin of the target weed species would also be important. As such, the strategy would be particularly suitable to weeds that have become invasive in foreign environments.

Many invasive weed species, including *S. hermonthica*, are allogamous and spread primarily by seed, and would thus be candidates for control by the sterile-female technique. Dioecious

targets would be particularly interesting, since a female-lethal rather than female-sterile construct could be employed, using promoters expressed early in development only in female plants to express a *barnase* or similar gene. This would remove the female portion of the transgenic target weed population, which would be desirable in the case of perennial targets such as weedy trees (Fig. 3). For any candidate, preliminary studies would likely be necessary to develop genetic manipulation protocols and identify suitable transposons, as well as hybridisation studies with close relatives in sympatry with the target population.[55] Tests of sterile-female technique concepts could be conducted in a model plant species such as *Arabidopsis thaliana* Heynhoe or *Nicotiana tabacum* L.

Other possible weed targets for control by the sterile-female technique could include: weeds that affect human health as well as agriculture; illicit crops; weeds that are invasive over large, difficult-to-manage areas; or weeds that are in taxonomic groups that are difficult to control owing to their close relationships with economically important plants. Target-selective chemical herbicides or specific biocontrol agents are often difficult to find for the latter targets, whereas the sterile-female technique would provide target-specific control. Risk of hybridisation between these target weeds and their related crops could be managed as described above (see Fig. 2), but in practice this risk could well be a moot point, given that seed for planting is unlikely to be collected from areas with infestations of these weeds. Outside the plant kingdom, possible targets could include many insect and other arthropod pests of agricultural, medical and veterinary importance, as well as other invasive species that reproduce sexually.
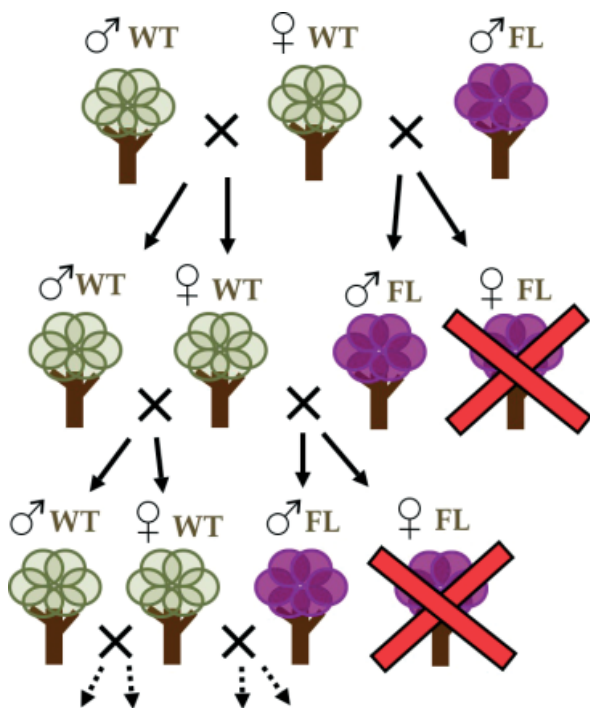

Figure 3. Suitability to dioecious targets. In a dioecious target species, only male transgenic individuals would be required to provide control. Thus, a female-lethal (rather than female-sterile) construct could be employed to remove transgenic female individuals from the population, reducing the overall pest pressure while the controlling construct is spreading through the target population. WT = wild type; FL = female lethal.

6 SYNTHESIS

Striga hermonthica brings ruin to subsistence farmers in its native range of sub-Saharan Africa, attacking many crop species and sometimes causing total crop loss.[1,56] It is allogamous (indeed, self-incompatible)[57] and reproduces only by seed. It is known to hybridise with a congener, *S. aspera* (Willd.) Benth., an occasionally weedy parasitic plant that occurs mainly on wild grasses.[58]

In order to target *S. hermonthica* for control by the sterile-female technique, it would be necessary to develop a standard protocol for transformation of this species. Transposable elements with activity within the *S. hermonthica* genome would need to be identified; they would also need to be capable of post-transformation transposition with a 'cargo' of embedded genes.[30] Promoters would be required that are specifically expressed within *S. hermonthica* female reproductive organs,[26] and induction systems would need to be tested. Initial tests of the concepts may be performed in conventional model plant species (e.g. *A. thaliana*, *N. tabacum*) or in the model parasitic plant *Triphysaria versicolor* (Frisch & CA Meyer).[56] Genetic resources are in development for the latter species, including an expressed sequence tag library that currently contains almost 50 000 entries[59] and another privately held library with over 9000 entries.[60]

The great potential benefit of controlling *S. hermonthica* would have to be weighed against the risk of completely eradicating the species by using the sterile-female technique in its centre of origin and the risk of the female-sterile construct passing to *S. aspera* (also in its centre of origin) and perhaps other close relatives. As a precaution, *S. hermonthica* germplasm could be collected from throughout its range prior to release of female-sterile plants. Prevention of hybridisation between female-sterile *S. hermonthica* and other *Striga* spp. could also be pursued, if desired.

A number of advances in both transgenic technology and biological knowledge of the *Striga* system would be required in order to bring this project to fruition. Clearly, this would be a costly and time-consuming challenge. However, in light of the destruction regularly caused by *S. hermonthica* and the potential for widespread control using the sterile-female technique, it might also be considered a sound investment. Once produced, female-sterile *S. hermonthica* plants would cost nothing to the farmers whom they would benefit and would be able to spread wherever the plant naturally occurs, no matter how remote. The return on the investment would come in the form of increased food security for the hundreds of millions of people living in contact with this weed, as well as the social and economic benefits this would engender both regionally and globally.

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